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# Biofilms On Porous Building Materials: Friend Or Foe?

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**Summary:** Current models generally view biofilms as having a detrimental role in the weathering process of building materials. The work covered by this paper will highlight that this may not be the complete story.

The degradation of many building materials is directly linked to the occurrence and movement of fluids within their porous structure, leading to secondary problems such as freeze-thaw damage, salt shattering, clay swelling, mineral leaching and precipitation. It is also thought that biofilm development on porous materials compounds this problem by absorbing water and acting like a wick in the transport of fluids from the external environment into the matrix of the building material.

However, experimental results indicate that biofilms can demonstrate hydrophobic properties, repelling water and consequently inhibiting the passage of water into the porous network of building materials. The water phase wettability of quartz was used to assess this characteristic.

The initial wettability of quartz fragments was determined using an environmental scanning electron microscope (ESEM). This showed the quartz fragments to be hydrophilic. The quartz fragments were then inoculated with microbes and incubated, facilitating biofilm development on their surfaces. The quartz fragments were re-examined using the ESEM, showing that quartz fragments coated in biofilm displayed hydrophobic behaviour. Hence, the addition of a biofilm to quartz can change the surface characteristics from hydrophilic to hydrophobic.

As quartz is the major component of sandstone this may help to understand the durability of sandstone undergoing water infiltration. Since biofilm development is usually associated with areas of building facades that are prone to water logging, the presence of the biofilm itself need not necessarily be a contributing factor to the problem. *In situ* biofilm may in fact impede ingress of external water sources into porous building materials by behaving as a water resistant layer. On the other hand the presence of a hydrophobic biofilm layer may add to the problem of water retention within porous building materials. Regardless of whether fluids are prevented from ingressing or egressing by hydrophobic biofilm barriers, either will have profound implications for the durability of porous building materials.

**KEYWORDS:** biofilm, wettability, sandstone, weathering, protection.

## 1 INTRODUCTION

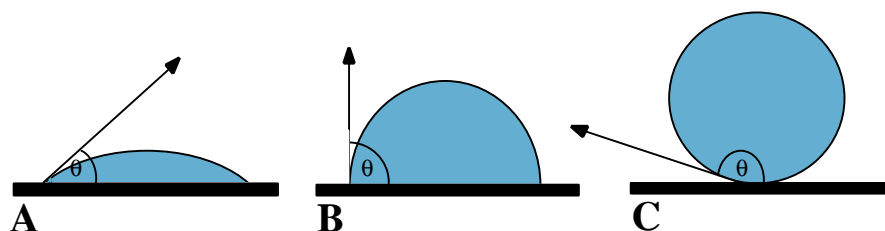
Previous research has shown that building materials, such as sandstone, limestone, granite, concrete and mortar, which are used in diverse climates, are readily colonised by microbes when the environment for growth is favourable. The microbes that have been studied include bacteria, cyanobacteria, fungi, lichens, and to a lesser extent algae (Paine *et al.* 1933, Milde *et al.* 1983, Palmer & Hirsch 1991, Urzi *et al.* 1991, Zhrebeyateva *et al.* 1991, Adams *et al.* 1992, De La Torre *et al.* 1993, Urrutia & Beveridge 1995, Jozsa *et al.* 1996, Wilimzig & Bock 1996, Ferris & Lowson 1997, Flores *et al.* 1997, Urzi & Realini 1998, Uchida *et al.* 1999).

Microbial-rock interactions are of considerable interest as microbes are thought to have a significant role in the degradation of building materials (Lyalikova & Petushkova 1991, Bock & Sand 1993, Hirsch *et al.* 1995a, b, Viles 1995, Barker & Banfield 1996, Palmer & Hirsch 1996, Feldman *et al.* 1997, Barker *et al.* 1998, Rogers *et al.* 1998, Papida *et al.* 2000). The problems associated with microbial attack include chemical and mechanical weathering. Chemical mechanisms include: dissolution,

precipitation, and alteration of mineral phases (Henderson & Duff 1963, Weed *et al.* 1969, Huang & Keller 1970, Lauwers & Heinen 1974, Halsey *et al.* 1995, Paris *et al.* 1995a, b, Spirakis 1996, Barker & Banfield 1998, Ehrlich 1998, Sweevers *et al.* 1998, Chigira & Oyama 1999; Labrenz *et al.* 2000, Liermann *et al.* 2000). Mechanical mechanisms include salt shattering, mineral expansion, freeze-thaw shattering, hydration-dehydration cycling and microboring (De La Torre *et al.* 1993, Halsey *et al.* 1995, Flores *et al.* 1997, Grondona *et al.* 1997, Wright 2000).

Microbes are also strongly associated with areas of high moisture retention such as areas of poor drainage. Once colonised in such areas they are thought to contribute to the problems associated with retention and or movement of moisture in the building material (Riley & Heiman 1996). However it has been demonstrated that biofilms can be a protective agent to building materials. An example of this was highlighted by Grondona *et al.* (1997) in their research surrounding the Casa Lis, a building in Salamanca in Spain which was built with Villamayor sandstone. In this instance, during restoration it was found that the sandstone that had developed an outer biofilm layer had suffered no underlying decay, whereas the sandstone that had no biofilm development was so severely altered that new stone was required to repair the damage. The preserved sandstone was apparently protected from salt crystal formation within the pores. Salt crystal formation within the pore space can cause salt shattering of the surrounding matrix. It is thought that the biofilm prevented evaporation of the fluid within the pore space, thus inducing a stable humidity that inhibited salt crystal formation. While the mechanisms at play in the biofilm are not fully understood, there is a high probability that the wettability characteristics may have had a significant role in preserving the sandstone.

The water phase wettability of a biofilm is an important factor effecting water placement and movement within porous building materials. Wettability describes the relative affinity of a fluid for a substrate (Walker 1999), and determines whether a fluid such as water will spread out across a substrate or be repelled and form discrete droplets. The degree of wettability is commonly measured by the contact angle that a droplet of fluid forms on a solid (Bascom 1992, Schoff 1992, Xie & Morrow 1998, Liu & Buckley 1999). In the case of water, when measured through the densest fluid phase, in this case the water itself, a low contact angle will be observed where the substrate is hydrophilic (Fig.1a), the water droplets form low domes, or occur as discrete sheets. Whereas, a high contact angle will occur on hydrophobic surfaces (Fig.1c), where water will occur in the form of discrete spherical droplets. Where a substrate is hydrophilic, it can be said to be water wet, which, under ambient conditions, is the case for most sandstones composed of predominantly quartz and feldspar (Barclay & Worden 2000). Whereas those that are hydrophobic, such as some calcitic limestones are regarded as oleophilic, or oil wet (Barclay & Worden 2000). The wettability of a biofilm will therefore affect the relationship between water and a substrate, either on, or within the porous building stone. Wettability will influence the biofilms ability to retain water, as well as affecting the combined biofilm and rock substrate's permeability, and thereby exert a control upon the location and movement of water within the biofilm-porous building material system. As many potentially damaging processes such as leaching, salt shattering and freeze-thaw shattering depend upon the presence of water, the relative water wettability of a biofilm will have major implications for the durability of porous building materials.



**Figure 1. Contact angles of water droplets, measured in a water vapour atmosphere, on a solid surface (e.g. quartz and biofilm). A) Low contact angle ( $< 90^\circ$ ), forming on an hydrophilic surface. B) Intermediate contact angle ( $\sim 90^\circ$ ), forming on a surface of intermediate wetness (neither hydrophilic nor hydrophobic). C) High contact angle ( $> 90^\circ$ ), forming on an hydrophobic surface.**

The wettability of any building material is a controlling factor upon the movement of water on its surface or within its pore spaces. The work highlighted in this paper will show that biofilms that grow on building materials can in fact alter the original wettability of that building material. In some instances this could be favourable and in others less so. The organisms studied include a mixed bacterial-fungal biofilm, an algal biofilm, and a lichen, all of which demonstrated different wettabilities.

## 2 Materials and Methods

### 2.1 Sample preparation

An analytical grade quartz crystal was placed on an oil free sterile surface and crushed with a sterile hammer. Thirty small chips approximately 2-3 mm in length were selected from the debris and stored in individual sterile Petri dishes. The chips were taken from the inner region of the crystal to ensure that only fresh, uncontaminated material was utilised. The quartz chips were handled at all times using sterile forceps to avoid contamination.

## 2.2 Initial wettability

Using a Peltier stage equipped Phillips XL30 ESEM with LAB<sub>6</sub> gun, and a 500 micron aperture Gaseous Secondary Electron Detector (GSED), the initial water phase wettability of each quartz chip was established.

The chips were pre-cooled in a standard refrigerator, placed on the Peltier stage in the ESEM chamber and maintained at 5°C. Wet mode was selected from the ESEM controller, and the chamber was pumped to 5 Torr. Upon attaining 5 Torr, the chamber was passed through up to five 'flooding' cycles from 5 to 10 Torr. Flooding with water vapour provides a suitable atmosphere for charge suppression, facilitates image amplification, maintains sample hydration, and most importantly to this experiment, supplies a wetting medium, water.

Chamber pressure was then increased to 6.5 Torr. At a pressure of 6.5 Torr and a temperature of 5°C, relative humidity reached 100%, and water condensed on to the surface of the sample. Observations of water droplet morphology (contact angle) were then recorded.

Where necessary (i.e. if flooding occurred), the whole process was repeated by pumping the chamber to 3 Torr, effectively sublimating all surface water, and returning to 6.5 Torr to repeat the procedure.

Images were acquired at a temperature of 5°C, a pressure of 6.5 Torr, a working distance of approximately 7.5 mm, an operating voltage of 20 kV, and a spot size of between 4 and 6.

Elemental analysis was carried out on each sample to ensure that the quartz chips used were completely pure. An Energy Dispersive X-ray Detector (EDX) was used at pressure 5 Torr and working distance 10 mm. X-rays were collected for 100 live seconds, with a count rate of approximately 1500 and a dead time of approximately 30%.

## 2.3 Biofilm development

In order to coat the quartz fragments with biofilm, a suitable inoculum was prepared. One 0.1 ml loop of each microbial culture was taken from 8 pure isolates of bacteria and one pure isolate of fungi. The loops of microbes were transferred into a conical flask containing 250 ml of Medium X (a growth medium composed of 50% nutrient broth and 50% malt extract broth). The microbial mixture was incubated at 25°C for 2 days to facilitate the development of a microbial consortium.

Using sterile forceps, 20 quartz fragments were individually transferred into twenty 100 ml conical flasks. Ten of the conical flasks contained 25 ml of distilled water and 10 ml of Medium X. Ten of the conical flasks contained 25 ml of distilled water. The flasks were sealed and sterilised in an autoclave for 15 minutes at 121°C. Once sterilisation was complete and the flasks cooled to room temperature 10 ml of the bacterial-fungal inoculum was added to the 10 flasks containing distilled water. This was carried out using strict aseptic technique. The remaining 10 flasks of distilled water and medium were left intact to provide a control, allowing any effects caused by the water and medium to be observed. All 20 flasks were incubated as above for 2 days; encouraging biofilm development on the 10 inoculated quartz chips.

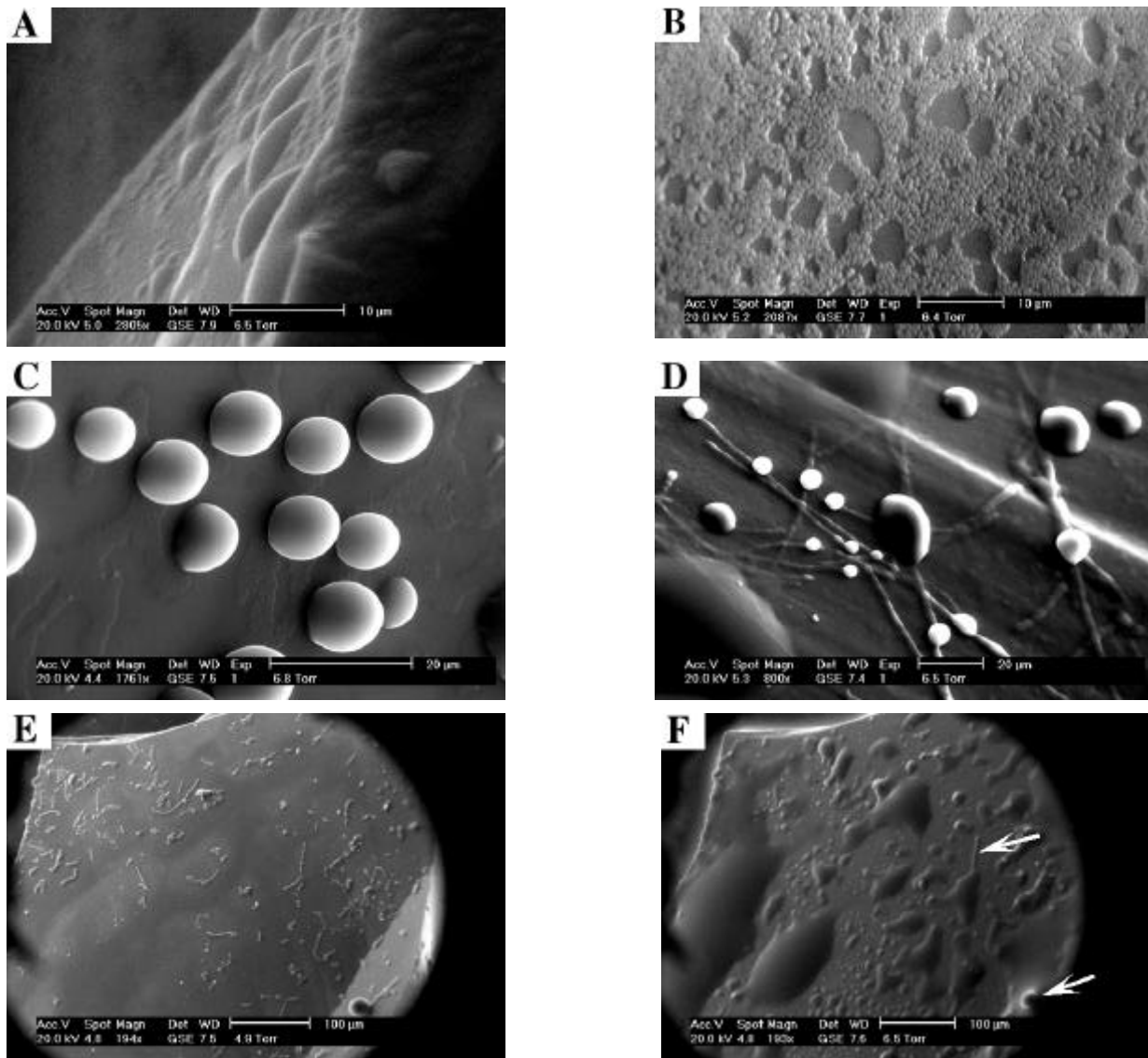
Following the incubation period the contents of each flask were emptied individually into separate sterile Petri dishes, allowing the quartz chips to be easily isolated.

For the algal biofilm analysis, the above process was repeated using 10 quartz chips, 5 for biofilm development and 5 for controls. The biofilm in this instance was composed of 3 axenic green algal cultures. The broth medium used was *Euglena Gracilis* Medium (EG) and Jaworski's Medium (JM) in a 1:1 ratio. The biofilm was cultured over 4 days at 16°C in an incubator set at a 16hr light / 8hr dark cycle.

For the lichen analysis a single sample of lichen was removed from a sandstone building and examined. The sample was not cultured.

## 2.4 Secondary wettability

The inoculated quartz chips and the control quartz chips were checked for the development of biofilm, and changes in wettability, using the same method of assessment as initial wettability. The results were imaged and stored using exactly the same method as for initial wettability. The lichen was examined under the same conditions as the biofilms.



**Figure 2.** A) Typical wettability of natural quartz surface, showing low domed hydrophilic droplets. B) Bacterial biofilm on quartz. C) High sphericity hydrophobic droplets on bacterial biofilm grown on quartz. D) High sphericity droplets and high domes, on hydrophobic bacterial-fungal biofilm. E) Quartz surface with algal biofilm. F) The same surface as in (E), displaying hydrophilic low domed drops of water. Two areas with algal cells indicated with arrows.

### 3 RESULTS

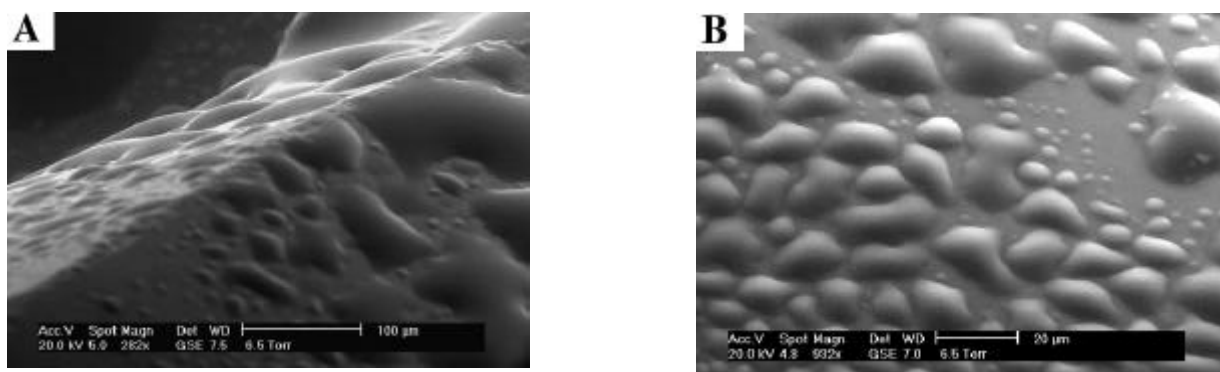
The results of the initial wettability assessment showed that all 30 quartz chips displayed hydrophilic properties and hence their wettability could be described as water wet. Water wetting was typified by low dome droplets displaying contact angles of considerably less than  $90^\circ$ , (Fig. 2a).

The results of the secondary wettability assessment showed that the 10 quartz chips exposed to the bacterial-fungal consortium successfully developed a biofilm (Fig. 2b). The wettability of the quartz chips coated in this biofilm changed from hydrophilic to hydrophobic, typified by high dome droplets with contact angles of  $> 90^\circ$ , (Figs 2c, d).

The 5 quartz chips exposed to the algal consortium successfully developed biofilms (Fig. 2e). The wettability of the quartz chips coated in algal biofilm remained hydrophilic, indicated by the low dome droplets of water on the algal surface (Fig. 2f).

The 15 quartz chips used as controls (i.e. those not exposed to microbes), retained their initial hydrophilic wettability (Figs 3a, b).





**Figure 3. Control samples: A) Quartz control sample from the bacterial-fungal experiment; B) quartz control from the algal experiment. Note both display low domed droplets of water and are hence still hydrophilic**

The EDX analysis confirmed that the quartz samples used were in fact pure  $\text{SiO}_2$  and the results were not due to inorganic contamination.

The lichen displayed mixed wettability (Fig. 4b).

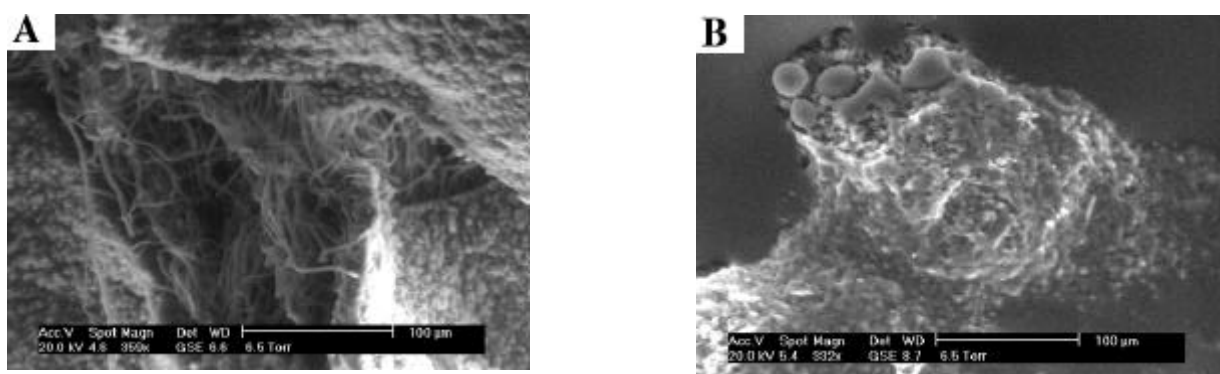
#### 4 DISCUSSION

The initial wettability of the 30 quartz chips was hydrophilic. This was the expected result, as quartz is widely recognised as such at ambient conditions (Barclay & Worden 2000). The secondary wettability of the 15 control chips remained unchanged, indicating that the water + Medium X broth and the water + EG:JM broth had no impact on the wettability of the quartz chips. Hence, it can be clearly stated that the secondary wettability changes in the inoculated samples were due to biofilm development and no other causes.

Of the 10 samples that underwent bacterial-fungal biofilm development, the wettability altered from hydrophilic to hydrophobic. This was most likely due to an interaction between the water and the bacterial-fungal cellular or extracellular material. The actual biomatter responsible and the mechanisms involved were not investigated, however, it is well known that hydrophobin, a fungal protein, has hydrophobic properties (Wosten *et al.* 1995).

Of the 5 samples that had algal biofilm development, the wettability remained hydrophilic. This indicated that the biomatter involved yielded a similar reaction to the water phase as the quartz.

The sample of lichen that was analysed showed a mixed wettability profile. The portion of the lichen that appeared to be fungal in nature demonstrated hydrophobic tendencies, the portion that appeared to be algal demonstrated hydrophilic tendencies. Given the nature of the biofilm wettabilities outlined above, this was not entirely unexpected.



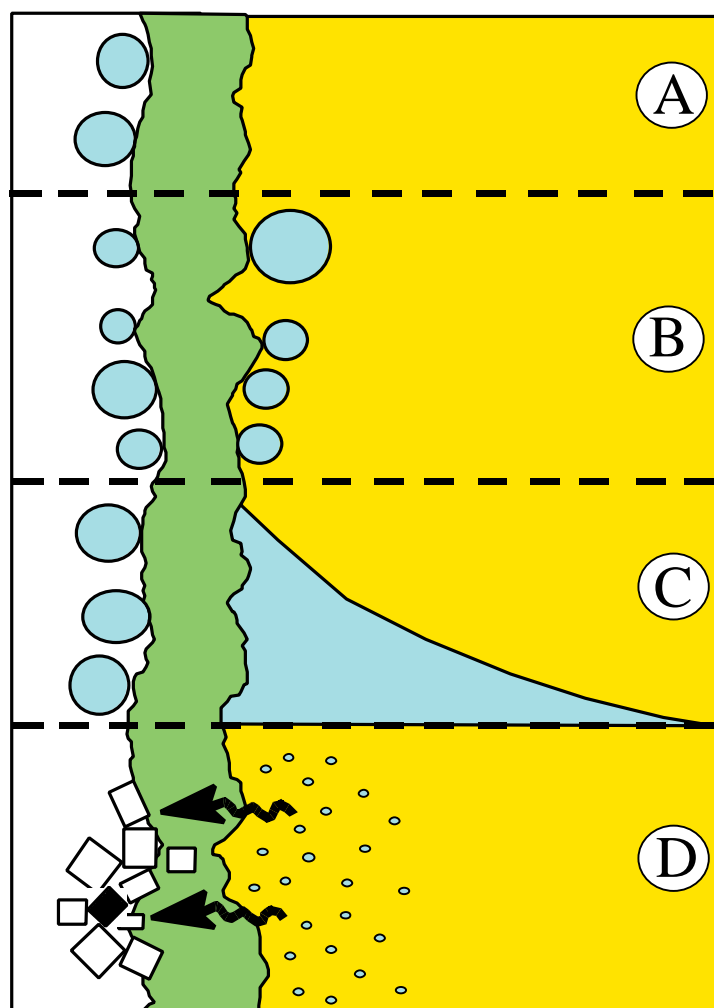
**Figure 4. A) Image of Lichen showing typical morphology, of 'fungal' hyphae and 'algal' cells. B) Typical variation in wettability across lichen. 'Fungal' hyphae (top left) are hydrophobic, indicated by spherical droplets of water, while 'algal' areas (bottom right) have sheets of water on them, typical of hydrophilic substrates.**

It appears to be quite clear that there is no general pattern of wetting characteristics that can be applied to microbes. The characteristics vary from kingdom to kingdom, and quite probably from species to species, therefore no broad assumptions can be made concerning the detailed relationships and interactions between biofilm and water. A biofilm may be predominantly hydrophilic, hydrophobic, or comprise a complex mixture of both.

The consortiums used in this research are quite artificial. In nature biofilms are complex three-dimensional networks comprising many different species of algae, bacteria, fungi and lichens (Bock & Sand 1993, Palmer & Hirsch 1996, Flores *et al.* 1997, Grondona *et al.* 1997). As this is so, they will therefore possess highly varied wettability characteristics, which will have many potential affects on the durability of porous building stone materials. A number of possible scenarios are briefly outlined below:

Hydrophobic biofilms, such as those produced by the bacteria and fungi examined during the present study, are likely to have an important role in controlling water migration through porous building materials. As neither bacteria nor fungi are restricted to the photic zone, they are at liberty to infiltrate deeply into building materials. Previous studies have indicated that bacteria in the presence of water can penetrate several centimetres into sandstone cores (Myers & McCready 1966, Myers & Samiroden 1967, Mills 1997). It is therefore feasible for such hydrophobic biofilms to develop over a much wider range of environments than algal biofilms.

Where an hydrophobic bacterial biofilm develops on the outer surface of a building stone, it could potentially act as a natural waterproofing to the building, possibly preventing ingress of water (Fig. 5a). This could reduce freeze thaw damage, salt shattering, and mineral dissolution and could leave the building stone in a less damaged condition than one without a biofilm *in situ*.



**Figure 5. Schematic section through a block of porous building material (sandstone), with a biofilm developed on the outer surface (left hand side). Key: yellow = sandstone, blue = water, green = biofilm. A) Biofilm with hydrophobic external surface. B) Biofilm with hydrophobic external and internal surfaces. C) Biofilm with hydrophobic external surface, and water ponded behind the internal surface. D) Biofilm with high humidity behind the internal surface, and salt crystals forming along the outer surface.**

However, if water is able to penetrate the structure from another direction (e.g. ingress from above, capillary rise, or possibly through condensation from within the building), it is equally possible that this water could be retained behind the biofilm (Fig. 5b). This is likely if the adhesive layer of the biofilm is hydrophobic. If this scenario occurred, the build up of water trapped behind the biofilm may accelerate the damage caused by mechanisms such as chemical leaching and frost shattering.

In extreme cases ponding of water could occur within the building stone and once again increase chemical leaching, clay swelling, salt shattering and freeze-thaw shattering (Fig. 5c).

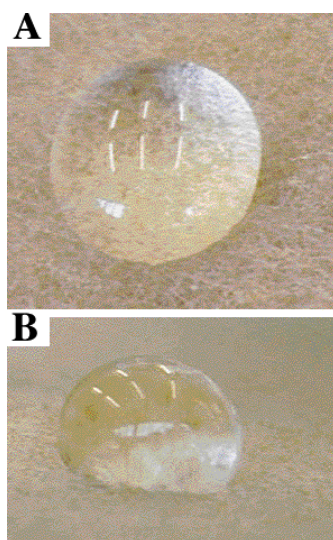
On a more positive note, it is also possible that a hydrophilic biofilm could act as a wick and transport dissolved salts to the surface of the biofilm where they can crystallise harmlessly (Fig. 5d), thus preventing salt shattering and eventual spalling. This has already been noted by Grondona (1997).

Hydrophilic biofilms (for example comprising predominantly of algae), may act as water wicks, retaining water within the biofilm and perhaps transporting it into the porous matrix. The impact of such biofilms, at least in terms of water penetration

into porous building materials such as sandstone is however likely to be limited. Most sandstones are already highly hydrophilic, being dominated by quartz, feldspar and clays such as illite, all of which are hydrophilic (Barclay & Worden 2000). Nevertheless, in cases where sandstone porosity is lined by more hydrophobic mineral cements such as calcite and dolomite, algal biofilm may have a more important role in the penetration of water into the substrate. The same will also be true for porous carbonates (such as oolitic limestone), where an algal biofilm could change pore walls from hydrophobic to hydrophilic. This would allow water to penetrate into building materials via the hydrophilic biofilm, thus bypassing pores that otherwise would have prevented or slowed down water movement through the pore space due to their hydrophobic nature. However, as algae are photosynthetic, such effects would be limited to the outer skin of any porous building material, and would therefore only effect the outer few millimetres of the building stone.

## 5 RECOMMENDATIONS

In order to assess the damaging or protective effects of the varying wettabilities of biofilms, each biofilm on a building should be examined with an open mind. A first approach to the interpretation of the biofilms wettability could be an *in situ* assessment achieved by applying small droplets of water to the biofilm. If this was not practical, a representative consortium could be grown on blocks of porous building material, under laboratory conditions, and observations made of single water droplets on the biofilm surface (Fig. 6). A second stage could involve a more detailed ESEM study of the natural biofilm, or the laboratory sample. Such an examination would determine the three dimensional architecture of the biofilm, elucidate the distribution of the various biofilm components, and define the wettability of each component present. The combined information could then be taken and used to model likely water migration pathways, and predict water distribution within the porous building material. The latter could then be used to predict likely mechanisms (involving water) that may affect the durability of that particular building material.



**Figure 6. A), B) Plan and side views of water droplets on a block of sandstone with a bacterial-fungal biofilm on the surface. Droplets have a high contact angle, being spherical in form, demonstrating the hydrophobic nature of the biofilm at the macroscopic scale. Droplets are approximately 2-3 mm in diameter.**

## 6 CONCLUSIONS

Biofilms of various types will readily colonise building materials. Analysis under ESEM has shown that the wettability of the underlying substrate can be dramatically altered by the presence of biofilm. The change can be towards either an hydrophilic or hydrophobic character. The implications for building materials can be either positive or negative. In some instances they can be protected from common weathering effects such as salt shattering and frost shattering, but in others the problems could be magnified. It is vitally important to assess the nature of biofilms present on buildings and monuments, just as important as assessing the properties of the building material. One must be sure if the biofilm is “friend or foe”.

## 7 ACKNOWLEDGEMENTS

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